Luminescent europium nanoparticles with a wide excitation range from UV to visible light for biolabeling and time-gated luminescence bioimaging†

Jing Wu,[a] Guilan Wang,[b] Dayong Jin,[c] Jingli Yuan,*[ab] Yafeng Guan*[a] and James Piper[c]

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Silica-encapsulated highly luminescent europium nanoparticles with a wide excitation range from UV to visible light (200–450 nm) have been prepared and used for streptavidin labeling and time-gated luminescence imaging of an environmental pathogen, *Giardia lamblia*.

Luminescent lanthanide complexes have been widely used as bio-labels for time-gated luminescence bioassays in recent years.[1] For most of visible-emitting lanthanide complexes, the sensitization mechanism generally involves a triplet pathway, achieved by energy transfer from a triplet excited state of organic ligands to the central lanthanide ions. The excitation window appears to be limited to the near-UV region (generally below 380 nm) due to the energetic constraints posed by the photophysics of sensitized lanthanide luminescence.[2] However, developing visible-emitting lanthanide bio-labels that can be sensitized by visible light has become an important task because the requirement for high-repetition pulsed UV excitation sources has been one of the major obstacles to the development of practical time-gated luminescence instruments. Substitution of UV-excited lanthanide labels by visible light-excited ones for time-gated luminescence bioassay is also advantageous since a number of biological systems are fragile to UV radiations.

It has been demonstrated that the excitation wavelengths for several Eu^{3+} complexes can be extended into the visible region with either the singlet pathway or usual triplet pathway mechanism.[3,4] Unfortunately, the use of these complexes as labels for efficient time-gated luminescence bioassay has been problematic because the singlet pathway of the complexes can only be stabilized in anhydrous non-coordinating organic solvents,[3] or the luminescence quantum yields of the complexes are relatively low (less than 10%).[4]

In the past decade, technology development of fluorescent nano-labels, such as quantum dots[5] and dye-doped silica or polyacrylonitrile nanoparticles,[6] has demonstrated many possible advantages over the traditional fluorophores. Additional benefits of fluorescent nanoparticles include their remarkable signal amplification and enhanced photostability since each particle-label contains a lot of luminescent molecules shielded by a coating layer. These properties enable them to be useful for monitoring real-time biological processes that need long-time continuous excitation over an extended period, as in conventional microscopy. Recently, several types of luminescent Eu^{3+} or Tb^{3+} complex-encapsulated nanoparticles, including polystyrene latex particles[7] and silica nanoparticles,[8] have also been developed for bioassays. These lanthanide nano-labels display an obvious advantage that strong interference of background noise can be easily eliminated using time-gated luminescence detection. However, the challenge posed by the requirement to excite in the UV (300–370 nm) has not been overcome.

Herein we describe a new generation of silica-based luminescent europium nanoparticles that can be excited over a wavelength range from UV to visible light (200–450 nm) in aqueous solution. Their utility as a luminescence label for time-gated luminescence imaging of an environmental pathogen, *Giardia lamblia* has been demonstrated.

The nanoparticles were prepared by a covalent binding-copolymerization method[8] using a visible-light-sensitized Eu^{3+} complex conjugated to an aminosilane, APS-CDHH-Eu^{3+}-DPBT conjugate (see Scheme S1 in ESI†), as a monomer precursor. The β-diketone ligand, 5-(4″-chlorosulfo-1′,1″-diphenyl-4′-yl)-1,1,1,2,2,3,3-heptafluoro-4,6-hexanedione (CDHH) has a chlorosulfonyl group that allows it to be able to covalently conjugate to 3-aminopropyl(triethoxyl)silane (APS) by the formation of an amide conjugation (–SO_2–NH–).[9] In the presence of Eu^{3+} and 2-(*N*,*N*-diethylanilin-4-yl)-4,6-bis(3,5-dimethylpyrazol-1-yl)-1,3,5-triazine (BPDT), the APS-CDHH conjugate can further form a luminescent ternary Eu^{3+} complex, $(APS\text{-}CDHH)_3Eu(DPBT)$, in anhydrous tetrahydrofuran.[3] After hydrolysis copolymerization of $(APS\text{-}CDHH)_3Eu(DPBT)$, free APS and tetraethyl orthosilicate (TEOS) in a water-in-oil (W/O) microemulsion initiated by aqueous ammonia, the silica-based luminescence nanoparticles were obtained. This method has two major advantages. One is that the hydrophobic visible-light-sensitized Eu^{3+} complex can be stably fixed in the particles without dye leaking during the washing, biolabeling and bioassay processes since the complex molecules are covalently bound to silicon atoms in the particles. The other is that free amino groups have been directly introduced to the nanoparticle's surface by using free APS in the nanoparticle preparation. These amino groups are directly available for

[a]*Department of Instrumentation & Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China. E-mail: jingliyuan@yahoo.com.cn; guanyafeng@dicp.ac.cn; Fax: +86-411-84706293 Fax: +86-411-84379590*
[b]*State Key Laboratory of Fine Chemicals, Department of Chemistry, Dalian University of Technology, Dalian, 116012, China*
[c]*Centre for Lasers and Applications, Division of Information and Communication Sciences, Macquarie University, NSW 2109, Sydney, Australia*
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biolabeling of the nanoparticles without tedious surface activation procedures.

It was found that the luminescence properties of the nanoparticles, especially their excitation spectrum, strongly depended on the composition of the W/O microemulsion. When hydroxyl containing organic solvents and surfactants, such as alcohols, Triton X-100 and CO-520, were used, the nanoparticles did not show the expected excitation spectrum at longer excitation wavelength (measured as around 335 nm). This phenomenon might be due to the singlet energy transfer pathway of visible-light-sensitized β-diketonate-Eu^{3+}-DPBT complexes[3] being prevented in a stronger coordinating solvent. The sensitive singlet energy transfer pathway of such complexes can be strongly affected by the interaction of the central Eu^{3+} ion with solvent molecules. In fact, the longer excitation wavelengths of β-diketonate-Eu^{3+}-DPBT complex dissolved in toluene disappeared when it was dissolved in an alcohol (see Fig. S1 in ESI†). Based on these results, we chose a microemulsion system consisting of dioctyl sulfosuccinate (AOT), heptane, toluene and a small amount of water to minimize the effect of the microemulsion on the luminescence properties of the nanoparticles.

The nanoparticles were characterized by transmission electron microscopy (TEM) and luminescence spectroscopic methods. As shown in Fig. 1, the nanoparticles are spherical and uniform in size, ~10 nm in diameter. Since the nanoparticles are very small with large specific surface area, they tend to link to each other under the irradiation of strong TEM electron beams.[10] Fig. 2 shows the time-gated luminescence spectra of the nanoparticles in a borate buffer and APS-CDHH-Eu^{3+}-BPDT conjugate in toluene. The toluene solution of APS-CDHH-Eu^{3+}-BPDT conjugate shows a maximal excitation peak at 406 nm, two middle excitation peaks at 297 and 342 nm, and a typical Eu^{3+}-complex emission pattern with a main emission peak at 615 nm ($^5D_0 \rightarrow {}^7F_2$) and several side emission peaks. It is noteworthy that, besides the usual maximal excitation peak of the Eu^{3+} complex at 335 nm, the buffer solution of the nanoparticles shows a wide excitation wavelength range extending to 450 nm with a peak at 384 nm. The ratios of the intensities of 384 to 335 nm and 406 to 335 nm are ~0.7 and ~0.5, indicating that the visible-light-sensitization

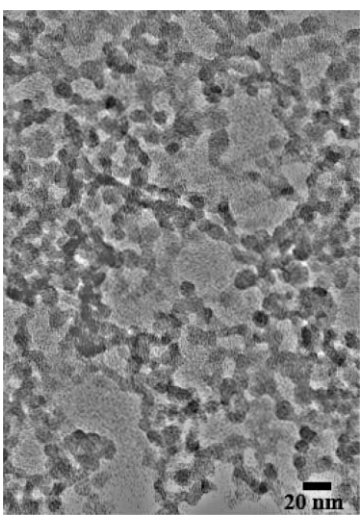

Fig. 1 TEM image of the nanoparticles at 200 000× magnification.

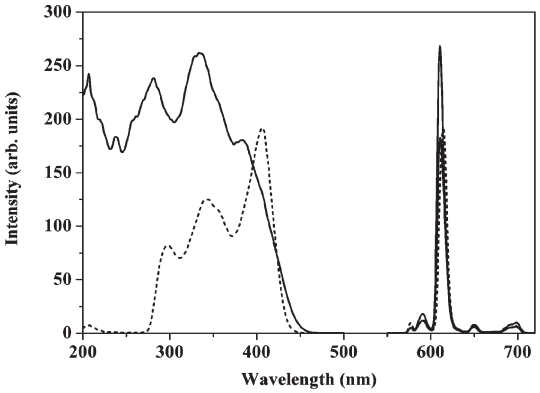

Fig. 2 Time-gated excitation and emission spectra of APS-CDHH-Eu^{3+}-DPBT conjugate (1.5 mg L^{-1}, dashed line) in toluene and the nanoparticles (8 mg L^{-1}, solid line) in 0.05 M borate buffer of pH 9.1.

property of the β-diketonate-Eu^{3+}-DPBT complex is still retained even in an aqueous phase after forming the nanoparticles. The luminescence lifetime and quantum yield of the nanoparticles in the borate buffer are measured to be 397 μs and ~21%, respectively. These results show that the nanoparticles are highly luminescent, and have a sufficiently long luminescence lifetime for long-delay time-gated luminescence measurement.

The protozoan parasite *Giardia lamblia* is an intestinal parasite in humans and animals, and has caused a number of diarrheal disease outbreaks worldwide.[11] One of the major sources of infection is through contaminated water. The intestinal parasite in various water sources is commonly detected by using immunofluorescent antibody (IFA) techniques.[12] However, the raw water samples, particularly after filtration and concentration, usually contain a large number of inert mineral particles and alga microorganisms with strong autofluorescence similar to those of immunofluorescently labeled protozoan cells. To improve detection selectivity for these target rare-event microorganisms, we have previously reported a time-gated luminescence microscope for high-contrast imaging of *Giardia lamblia* in complex environmental water samples using a UV-excited luminescent Eu^{3+} complex as a label.[13] To demonstrate the utility of the new nanoparticles which can be excited with either UV or visible light, the nanoparticle-labeled streptavidin (SA) was prepared using an established BSA (bovine serum albumin) coating method[8] (its reactivity to biotinylated antibody was confirmed by using it for a time-resolved fluoroimmunoassay of human prostate-specific antigen, see ESI†), and used for time-gated luminescence imaging of *Giardia lamblia* in complex environmental water samples.

Fig. 3 shows the bright-field, luminescence (excited with 330–380 nm and 380–420 nm, respectively) and time-gated luminescence (excited with 330–380 nm, another image excited with 380–420 nm is shown in Fig. S4 of ESI†) images of *Giardia* cysts that have been incubated with the anti-*Giardia* antibody, biotinylated secondary antibody and the nanoparticle-labeled SA, in the water samples containing green algae and inert particles, respectively. Without time-gated detection, specific identification of single *Giardia* cysts from strongly autofluorescent green algae and inert particles (appearing red under excitation) is very difficult, whereas the time-gated luminescence images show only the specific luminescence signals from the *Giardia* cysts. These results

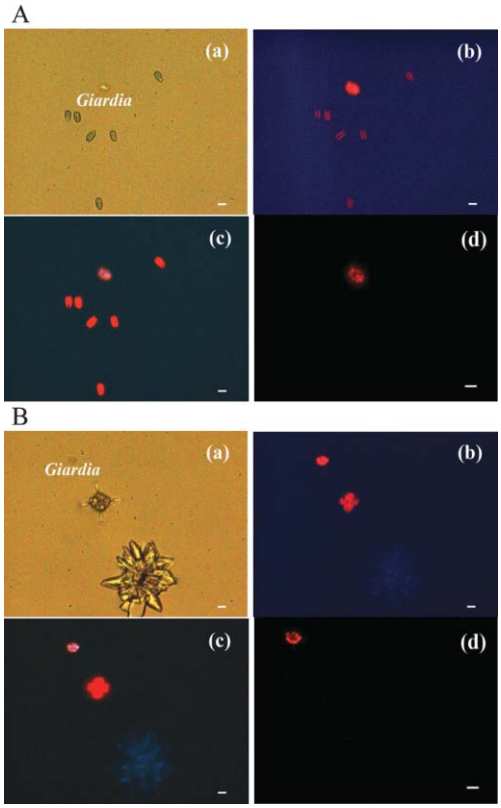

Fig. 3 Bright-field (a), luminescence (b, c, excited with 330–380 nm and 380–420 nm, respectively) and time-gated luminescence (d, excited with 330–380 nm) images of *Giardia lamblia* stained by the nanoparticle-labeled SA in water samples containing green algae (A) and inert particles (B). Scale bars, 10 μm. The time-gated luminescence images are shown in pseudo-color (wavelength of 615 nm) treated by a SimplePCI software.[15]

demonstrate that the time-gated luminescence imaging technology using the as-prepared nanoparticles as a label can substantially eliminate the interference of short-lived fluorescence from the other non-target co-existing substances, to provide a highly specific and sensitive detection for environmental microorganisms. The clear images excited with 380–420 nm (Fig. 3 and Fig. S4, ESI†) also demonstrate that the nanoparticle-based luminescence label prepared in this work has a practical value in time-gated luminescence bioassays for extending the excitation spectrum into the visible region. This unique visible-light-sensitized property offers an opportunity for a broader range of time-gated luminescence bioassay applications (*e.g.*, the visible-light-excited time-gated luminescence imaging to observe living cells and organisms) and improving the design of current time-gated luminescence instruments (*e.g.*, using solid-state LEDs or laser diodes with maximum outputs over 370 nm instead of a xenon flash lamp as excitation source. These sources offer significant advantages in much smaller size, lower cost and higher excitation efficiency[14]). The result of a control experiment in the absence of the anti-*Giardia* antibody reveals that the non-specific binding of the nanoparticles on *Giardia* cysts does not occur in the absence of the anti-*Giardia* antibody (see Fig. S5 in ESI†).

In summary, silica-encapsulated highly luminescent europium nanoparticles with a unique property in aqueous phase, a wide excitation range from UV to visible light, have been prepared and used for highly specific time-gated luminescence imaging of *Giardia lamblia*. The results indicate the practicability of the new nanoparticles for time-gated luminescence imaging of living cells or tissues in life science. This label could also be expected to promote the application and instrumentation of time-gated luminescence bioassay technology.

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